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# Impact of Two Compounds of Metal Organic Frameworks on Some Physiological Activities and FTIR Investigation of *Culex pipiens* Larvae (Diptera: Culicidae)

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**ABSTRACT**

Mosquitoes are considered the main vector for several diseases. Fortunately, numerous strategies have been adopted to control mosquitoes. However, traditional insecticides have hazardous impacts on the environment and living organisms. Thus, the present study attempted to discover new compounds for using as alternatives to traditional insecticides with the same potency. In this study, two metal organic framework compounds were evaluated as larvicidal against *Culex pipiens*. In addition, their effect on some physiological parameters was addressed by using FTIR technique. The results revealed significant differences (increasing or decreasing) in all tested parameters in both treatments; however, Fe-MOF was more effective than Mn-MOF during all stages for larvae after application. It was concluded that, given their acute and chronic effects, these compounds can be used in the control program against mosquitoes.

## INTRODUCTION

The mosquito *Culex (Cx) pipiens* is a worldwide insect causing dreadful nuisance and transmitting many dangerous *Cx. pipiens* diseases. In Egypt, it is the main vector of filarial worm *Wuchereria bancrofti* **(Joseph *et al*., 2011)** as well as Rift Valley fever virus **(El-Zayyat *et al*., 2017)**.

Mosquito control has become increasingly difficult in Egypt due to the emerging resistance of numerous insecticides **(Zayed *et al*., 2006 )**. The large-scale employment of reliable control strategies to be used against arthropod pests and vectors is a key challenge for current entomology and parasitology **(Benelli & Mehlhorn, 2016; Benelli & Beier, 2017; Benelli & Duggan, 2018)**. Nevertheless, the massive overuse of chemical pesticides led to fast-growing development of resistance in targeted arthropods and severe consequences for human health and the environment **(Naqqash *et al*., 2016; Desneux *et al*., 2007)**. Notably, the failure of control programs, coupled with the fast spread of highly invasive arthropod vectors has led to a rise of various arthropod-borne diseases—as outlined by the recent Zika virus outbreaks in the Americas and the Pacific **(Fauci & Morens, 2016; Yakob & Walker, 2016; Benelli & Romano, 2017)**, with

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dramatic consequences for the public health. To face this challenge, researchers focused on novel and potentially safe control tools **(Stevenson *et al.*, 2017; Benelli & Pavela, 2018; Wilke *et al.*, 2018)**.

Few major biomarker enzymes were evaluated to investigate the effects of botanical biocides in the insect resistant developed enzymes; namely, esterases, phosphates, glutathione-S-transferase, monooxygenase, etc. **(Jones & Bancroft, 1986)**.

Therefore, this experiment was carried out to assess the effect of MOF nanoparticle on physiological aspect using a novel technique to confirm the enzymatic changes recorded upon substituting the traditional pesticides with a novel effective material to control mosquitoes.

## MATERIALS AND METHODS

### 1. Tested insect

#### *1.1. Culex pipiens culture*

The larvae of *Culex pipiens* were obtained from the Medical Entomology Institute. Self-perpetuating colonies were maintained and created in the incubator rooms of the Research and Training Center, Ain- Shams University. Rearing method was applied as described in the study of **Farag *et al*. (2021**). Biochemical studies were carried out on late third larval instar.

**1.1.1. Sub lethal effect of metal-MOFs on *Culex pipiens* larvae.** The sub lethal concentration was determined via applying a serial of concentrations of the third larval instar, with a given mortality percentage between 20 & 90, as mentioned in the work of **Kamel and Abdel-Hameed (2023)**.

### 2. Biochemical activities

Tissue samples of tested groups of the 3$^{rd}$ instar larvae of *Cx. pipiens*, treated for 48 hours with the determined LC50 (Fe and Mn MOF nanoparticle, provided by **Kamel and Abdel-Hameed (2023)**, were subjected to an evaluation for their effect on some physiological parameters.

#### *2.1. The total protein*

Measurement of total protein content was achieved by using the technique of Folin-Ciocalteu **(Lowry *et al.*, 1951)**.

#### *2.2. Total lipid*

The estimation of total lipid was done quantitatively according to **Knight *et al.***, **(1972)**, using phospho vanillin reagent.

### *2.3. Total carbohydrate*

Total carbohydrate value was determined via applying the method of **Singh and Sinha (1977)**.

### *2.4. Acid phosphatase activity (ATPase)*

The activity of acid phosphatase (ATPase) was measured in untreated and treated tissue samples **(Laufer & Schin, 1971)**.

### *2.5. Glutathione S-transferase (GST) activity*

The calculation of glutathione S-transferase activity was estimated for each tested nanoparticle compound according to the methods used briefly in the study of **Shahat *et al.* (2020)**.

### 3. Evaluation of Fe and Mn MOF nanoparticle effects via FTIR technique

After applying the tested materials on 3rd larval instar for 48hrs, the larvae were gently washed and transferred to clean pones. When larvae completed their life cycle, samples were collected, with males and females for investigating by FTIR. Furthermore, IR spectra were carried out on JASCO FT/IR 6100 Japan spectrometer (National Research Centre, Egypt) using KBr discs.

### 4. Statistical analysis

The experimental data were recorded using standard procedures. An analysis of variance and a multiple comparison test were performed with the data expressed as (mean ± S.D). According to the Tukey test at $P < 0.05$, statistical values that are separated by the same letter are not significantly different (one-way ANOVA). The statistical analyses were carried out using Minitabe version 17.

## RESULTS

### 1. Biochemical activities

The results displayed in Table (1) reveal that the treated larvae were affected in the tested parameters. Protein values showed an increase in both treatments of Mn & Fe-MOF, compared to the control. Moreover, the lipid exhibited an increase in the treated larvae. Hence, carbohydrate and ATPase decreased in both treatments in comparison with the control. There are significant differences by increasing or decreasing in each treatment, compared to the control for all tested biochemical parameters. Additionally, a significant difference was detected in the activity of GST, noting an increased value, compared to the control samples. While, the *F*- values recorded a degree of significance in all tested parameters, compared to the control. Figs. (1, 3, 5, 7, 9) clarify the individual confidence among the means of all tested compounds (among each other) and those of the control. In addition, Figs. (2, 4, 6, 8, 10) illustrate the interval plot of means' level.

The increasing and decreasing taken place in the treated larvae are remarkably obvious, compared to the control

**Table 1.** Effect of tested nanoparticle compounds on selected biochemical parameters content for *Cx. pipiens* third larval instar

| Tested parameter | Control Mean±SD | Fe-MOF Mean±SD | Mn-MOF Mean±SD | *P*- value | *F*- value |
|---|---|---|---|---|---|
| Total protein (mg/gm.b.wt) | 5.61±0.04b | 4.38±0.03c | 7.23±0.03a | 0.000 | 15507.05 |
| Total lipid (mg/gm.b.wt) | 2.58±0.03a | 1.87±0.02c | 2.40±0.03b | 0.000 | 1955.62 |
| Total carbohydrate (mg/gm.b.wt) | 3.53±0.02a | 2.91±0.01c | 3.33±0.02b | 0.000 | 2953.61 |
| ATPase activity (mg/gm.b.wt) | 1.42±0.01a | 0.50±0.01c | 1.15±0.03b | 0.000 | 7034.68 |
| Glutathione S-transferase activity (mg/gm.b.wt) | 2.72±0.01c | 5.75±0.13b | 7.78±0.14a | 0.000 | 4731.35 |

Rows with different letters have significant differences between them in an ascending way.

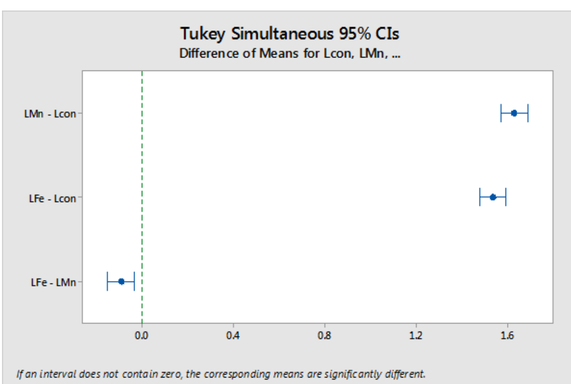


**Fig. 1.** Individual confidence between means of Fe & Mn-MOF application on *Cx .pipiens* third larval instar (with each other) and control for total protein of larvae

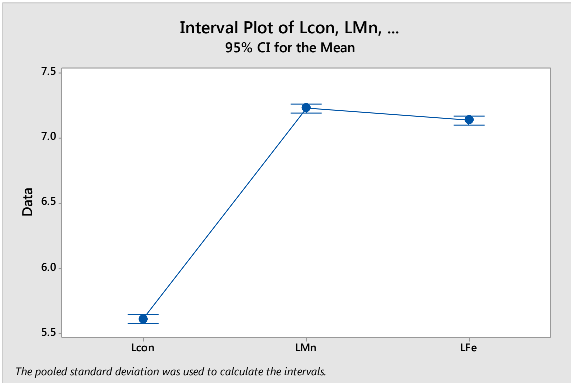


**Fig. 2.** Interval plot of level means of Fe & Mn-MOF application on *Cx. pipiens* third larval instar (with each other) and control for total protein of larvae

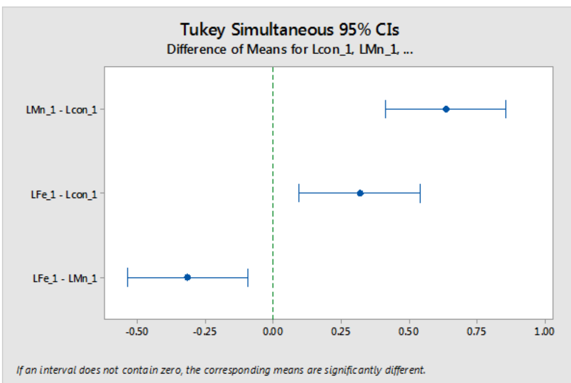


**Fig. 3.** Individual confidence between means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar (with each other) and control for total lipid of larvae

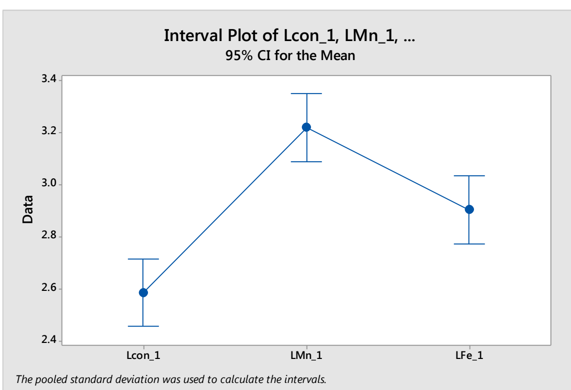


**Fig. 4.** Interval plot of level means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar (between each other) and with control for total lipid of larvae

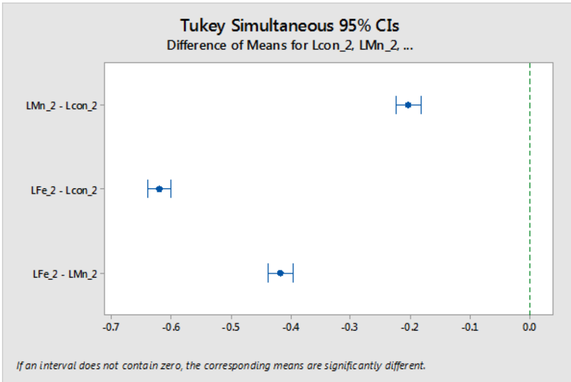


**Fig. 5.** Individual confidence between means of Fe & Mn-MOF application on *Cx. pipiens* third larval instar (with each other) and control for total carbohydrate of larvae

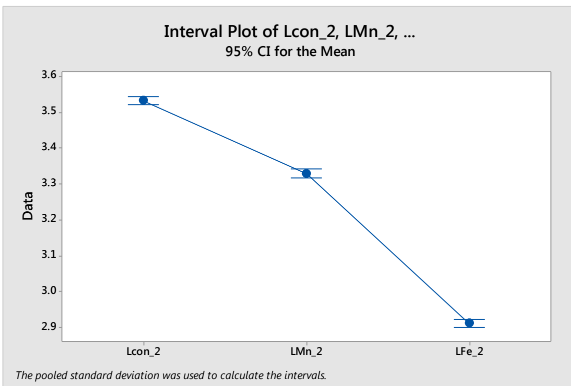


**Fig. 6.** Interval plot of level means of Fe & Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for total carbohydrate of larvae

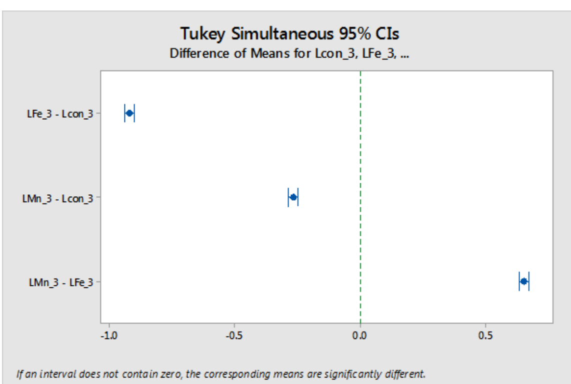


**Fig. 7.** Individual confidence between means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for ATPase activity of larvae

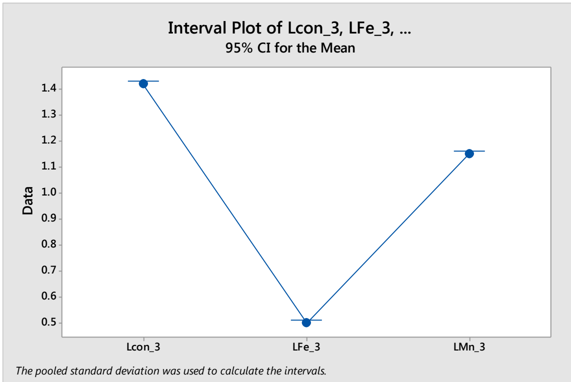


**Fig. 8.** Interval plot of level means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for ATPase activity of larvae

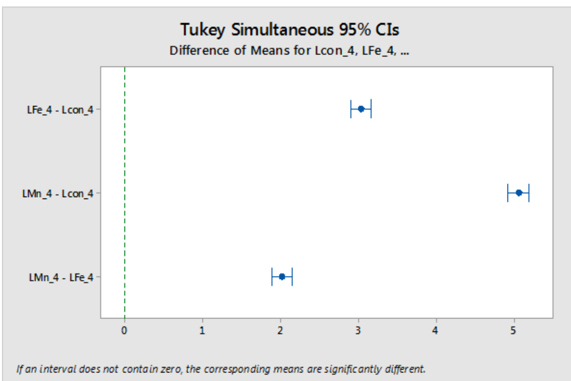


**Fig. 9.** Individual confidence between means of Fe & Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for GST activity of larvae

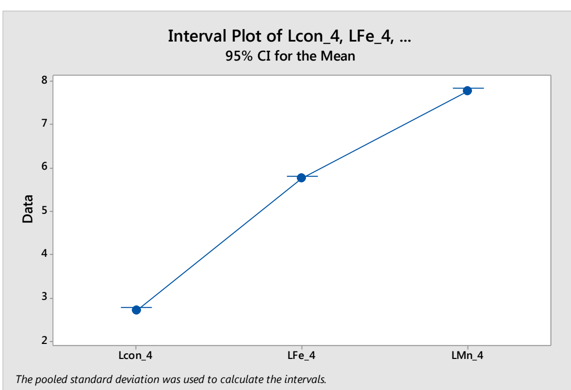


**Fig. 10.** Interval plot of level means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for GST activity of larvae

There is no overlapping between means that indicate the presence of statistically different significance in them. Results presented in Table (2) show the difference in means of tested biochemical parameters compared to the control group in male stage of mosquito. Significant differences were detected in total protein for both treatments between each other and with the control in a decreasing root. In addition, the results revealed significant difference in carbohydrate by increasing in all treatments; while in Fe-MOF, it was more than in Mn-MOF. In case of ATPase and GST, a significant difference was recorded by decreasing and increasing values in comparison with each other and the control group. In case of total lipid, the significant difference by decreasing appeared between control and both treatments. Figs. (11- 20) show the significant differences in both treatments between each other and with the control's means, except for the case of total lipid, which showed significant difference with the control, with no differences between both treatments.

**Table 1.** Effect of tested nanoparticle compounds on selected biochemical parameters content of male stage for *Cx. pipiens*

| **Tested parameter** | **Control Mean±SD** | **Fe-MOF Mean±SD** | **Mn-MOF Mean±SD** | ***P*- value** | ***F*- value** |
|---|---|---|---|---|---|
| Total protein (mg/gm.b.wt) | 6.04±0.06a | 5.83±0.02b | 5.60±0.01c | 0.000 | 268.98 |
| Total lipid (mg/gm.b.wt) | 2.71±0.02a | 2.47±0.02b | 2.55±0.01b | 0.000 | 16.14 |
| Total carbohydrate (mg/gm.b.wt) | 3.88±0.02b | 6.51±0.3a | 4.00±0.02b | 0.000 | 2203.78 |
| ATPase activity (mg/gm.b.wt) | 1.71±0.01a | 1.18±0.02c | 1.26±0.03b | 0.000 | 2126.04 |
| Glutathione S-transferase activity (mg/gm.b.wt) | 3.08±0.01c | 8.80±0.01b | 8.89±0.01a | 0.000 | 83976.93 |

Rows with different letters have significant differences between them in an ascending way.

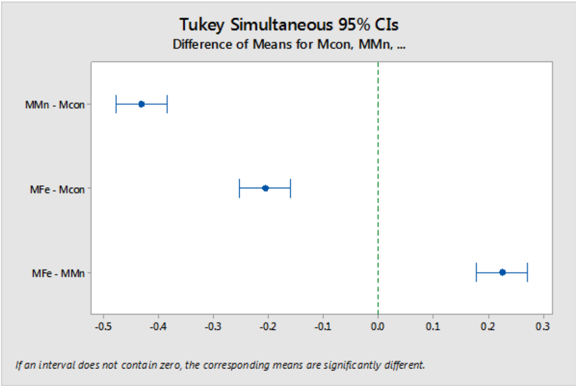


**Fig. 11**. Individual confidence between means of Fe & Mn-MOF application on *Cx. pipiens* third larval instar with each other & with control for total protein of adult male

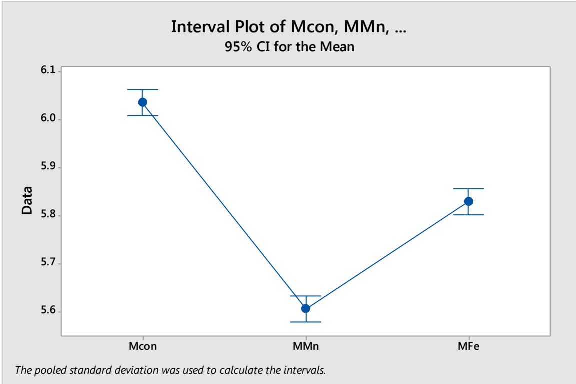


**Fig. 12.** Interval plot of level means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for total protein of adult male

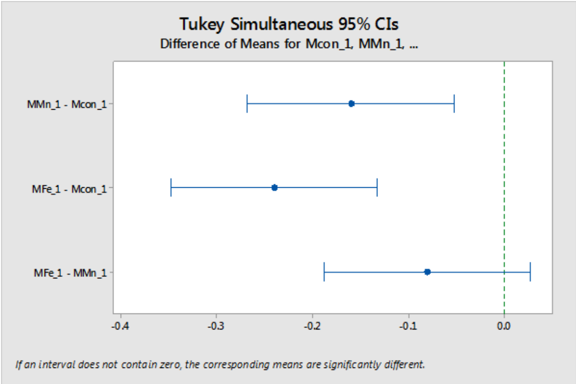


**Fig. 13.** Individual confidence between means of Fe & Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for total lipid of adult male

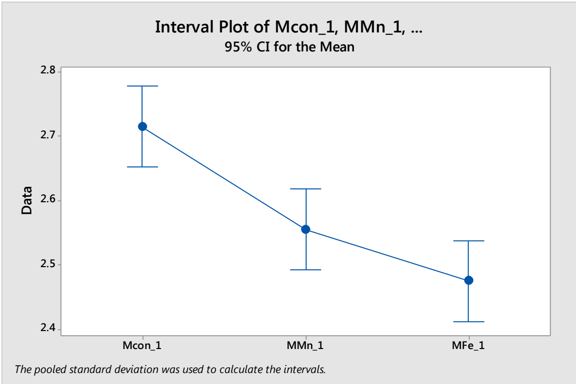


**Fig. 14.** Interval plot of level means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for total lipid of adult male

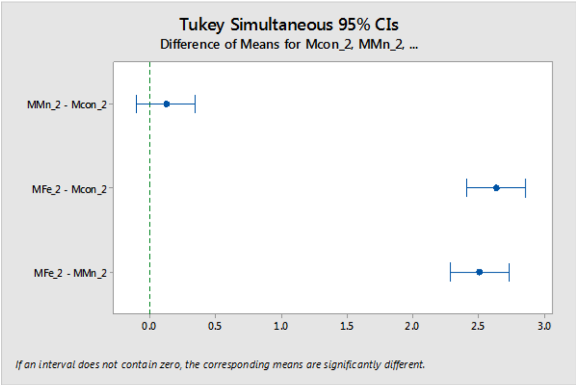


**Fig. 15.** Individual confidence between means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for total carbohydrate of adult male

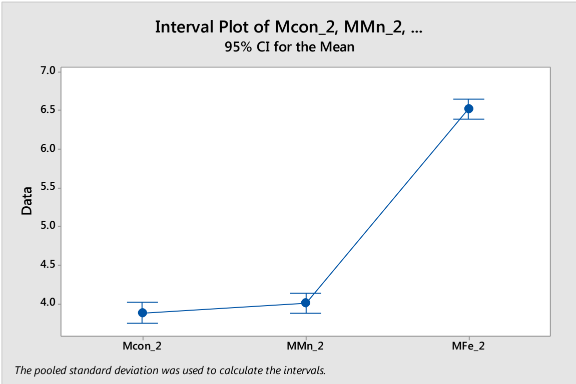


**Fig. 16.** Interval plot of level means of Fe & Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for total carbohydrate of adult male

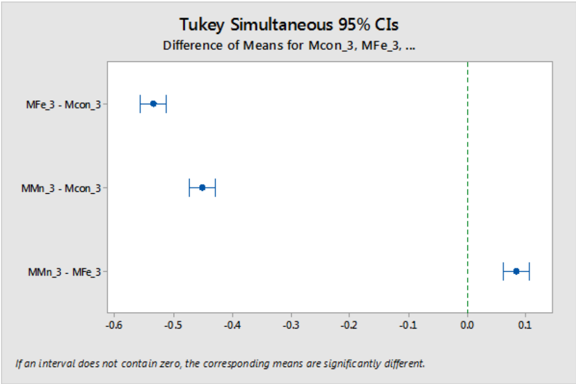


**Fig. 17.** Individual confidence between means of Fe & Mn-MOF application on *Cx .pipiens* third larval instar with each other and with control for ATPase activity of adult male

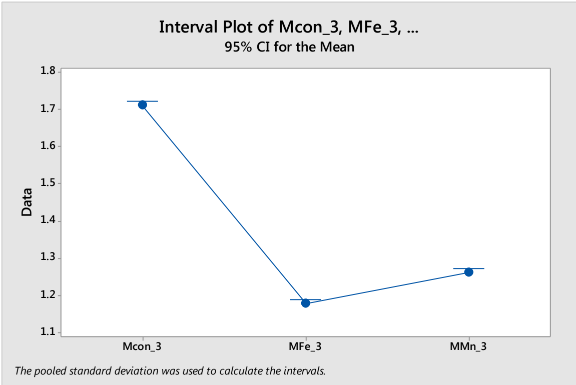


**Fig. 18.** Interval plot of level means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for ATPase activity of adult male

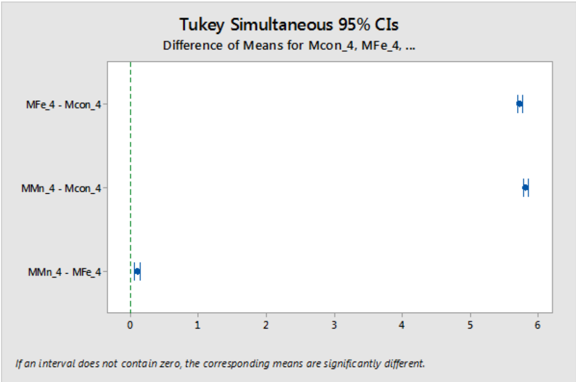


**Fig. 19**. Individual confidence between means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for GST activity of adult male

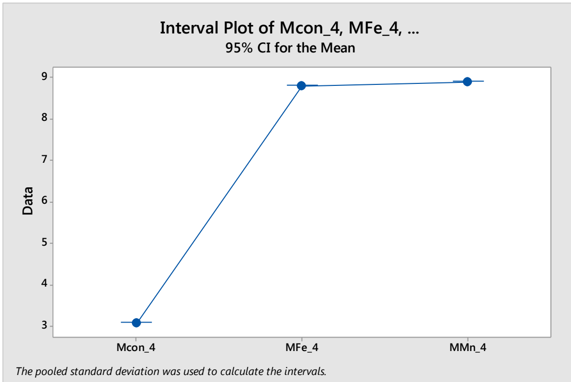


**Fig. 20.** Interval plot of level means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for GST activity of adult male

The female stage of mosquito showed a significant difference for all parameters examined by decreasing values, compared to the control while increasing values were only detected in GST upon comparing with the control, as shown in Table (3). The treatment with Fe-MOF compound showed highly significant differences more than Mn-MPF compound (Figs. (21- 30) display these relations clearly).

**Table 3.** Effect of tested nanoparticle compounds on selected biochemical parameters content of female stage for *Cx. pipiens*

| **Tested parameter** | **Control Mean±SD** | **Fe-MOF Mean±SD** | **Mn-MOF Mean±SD** | ***P*- value** | ***F*- value** |
|---|---|---|---|---|---|
| Total protein (mg/gm.b.wt) | 6.12±0.02a | 5.86±0.03b | 5.60±0.01c | 0.000 | 1419.61 |
| Total lipid (mg/gm.b.wt) | 3.08±0.05a | 2.24±0.02c | 2.65±0.02b | 0.000 | 1438.40 |
| Total carbohydrate (mg/gm.b.wt) | 3.92±0.01a | 3.41±0.01c | 3.51±0.02b | 0.000 | 492.14 |
| ATPase activity (mg/gm.b.wt) | 1.78±0.01a | 1.23±0.02c | 1.31±0.02b | 0.000 | 2958.54 |
| Glutathione S-transferase activity (mg/gm.b.wt) | 3.24±0.02c | 8.90±0.01b | 8.93±0.02a | 0.000 | 380534.29 |

Rows with different letters have significant differences between them in an ascending way.

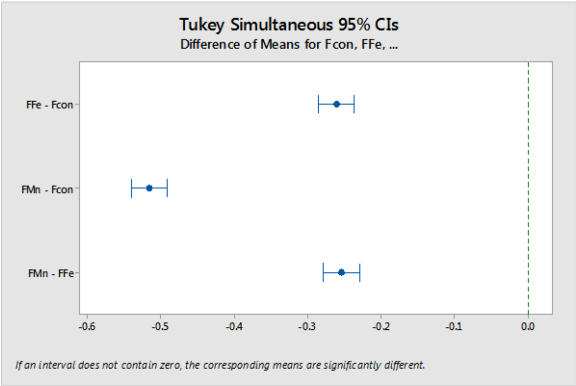


**Fig. 21.** Individual confidence between means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for total protein of adult female

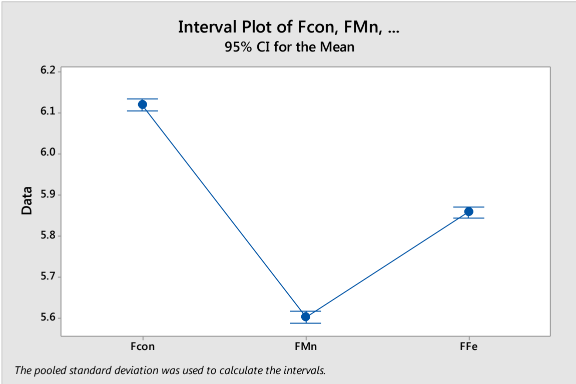


**Fig. 22.** Interval plot of level means of Fe & Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for total protein of adult female

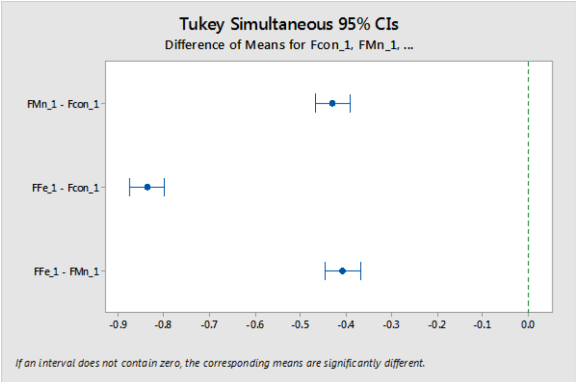


**Fig. 23.** Individual confidence between means of Fe & Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for total lipid of adult female

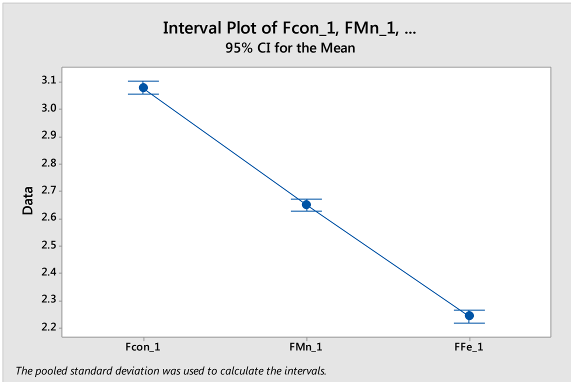


**Fig. 24.** Interval plot of level means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for total lipid of adult female

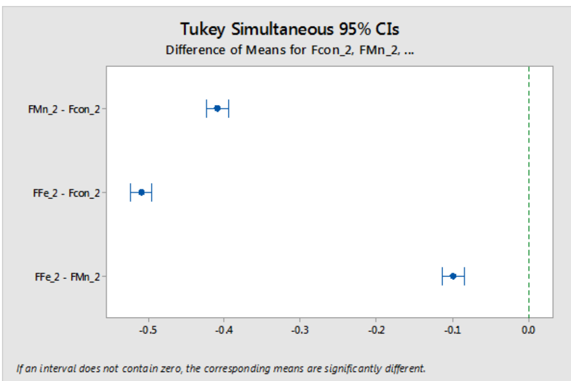


**Fig. 25.** Individual confidence between means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for total carbohydrate of adult female

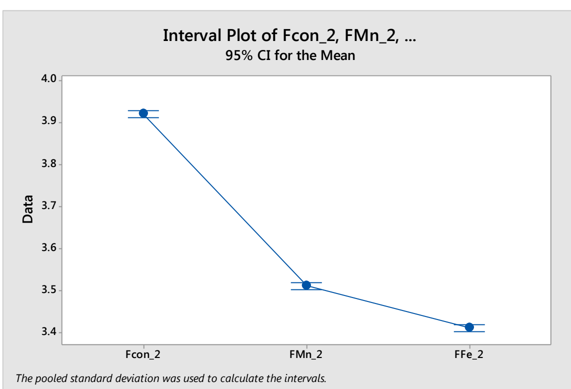


**Fig. 26.** Interval plot of level means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for total carbohydrate of adult female

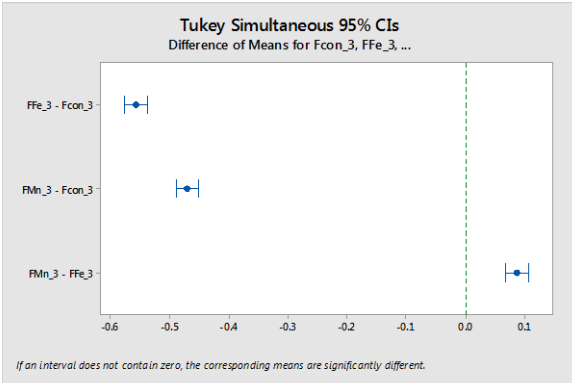


**Fig. 27.** Individual confidence between means of Fe & Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for ATPase activity of adult female

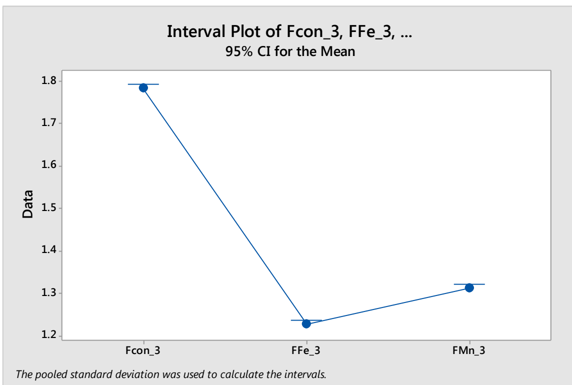


**Fig. 28.** Interval plot of level means of Fe & Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for ATPase activity of adult female

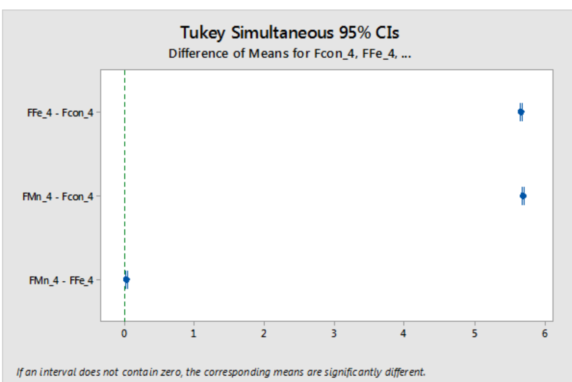


**Fig. 29.** Individual confidence between means of Fe & Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for GST activity of adult female

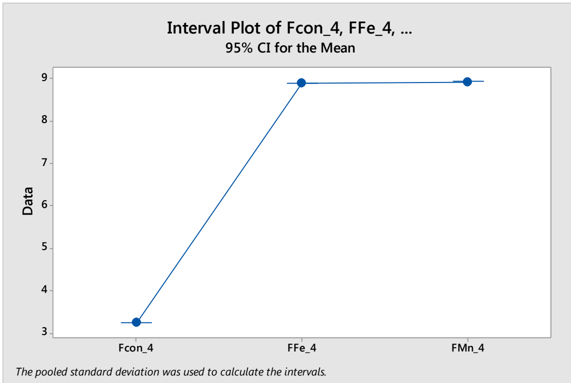


**Fig. 30.** Interval plot of level means of Fe &Mn-MOF application on *Cx. pipiens* third larval instar with each other and with control for GST activity of adult female

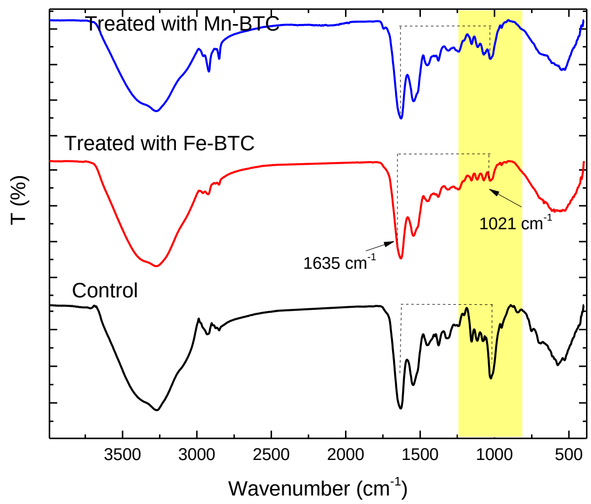


**Fig. 31.** FTIR for control and treated larvae of *Cx. pipiens* with Fe & Mn-MOF

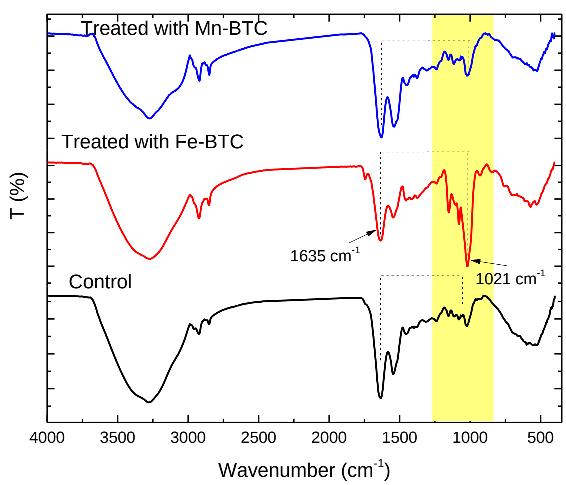


**Fig. 32.** FTIR for control and treated male of *Cx. pipiens* with Fe & Mn-MOF

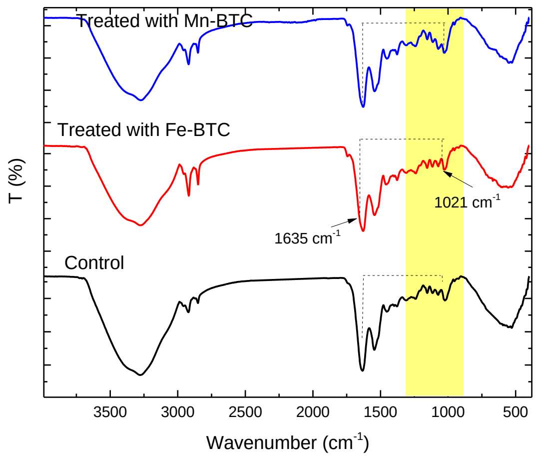


**Fig. 33.** FTIR for control and treated female of *Cx. pipiens* with Fe & Mn-MOF

## DISCUSSION

Researchers have focused to develop nano-scale compounds as an alternate control agent for mosquito larvae. The new field of nanoparticles applications was studied by many authors **(Abdelhameed *et al.*, 2017)** using nanoparticle on fabric against adult mosquitoes and (**El-Sayed *et al.*, 2020)** applying AgNPs on larvae and adult. While, in the current study, an attempt was made to figure out their effects on some biological aspects to clarify their mortality action on mosquito larvae. Silver nanoparticles cause toxic effects and may be accompanied with specific biological system **(Kumud *et al.*, 2013)**. The effect of decreasing total protein, lipid, carbohydrate ATPase and increasing GST activity is perhaps attributed to the increase in oxidative stress, which agrees with the finding of **Wong and Jim (2017)**. The significant difference of treated samples and control in protein, lipid, carbohydrate, ATPase and GST probably occurred as a result of cell membrane damage or intracellular penetration and damage or oxidative stress, these effects may occur individually or combined with each other **(Ragheb *et al.*, 2020; AlSalih *et al.*, 2021)**.

Based on the results of FTIR, it was observed that, two main groups were recorded at wavelength 1021 (glucosidic ring) and 1635 (carbonyl group). Figs. (31, 33) show a decrease in carbohydrate group at a ratio of 1: 2, in comparison with carbonyl group (i.e. protein and lipid) for larvae and adult female mosquito in both treatments. This observation may be related to the ability of MOFs to capture the carbohydrate compounds **(Efwita *et al.*, 2019)**. The necessity for carbonyl groups during metamorphosis and reproduction may have caused an increase in the ratio of carbonyl groups in adults **(Arrese & Soulages, 2011; Hou *et al.*, 2015)**. Despite the increase recorded in carbonyl group at adult stages, males treated with Fe-MOF (Fig. 32) showed that the carbohydrate group witnessed a rate of increase more than that recorded for the carbonyl group. This finding may be due to the degradation of Fe-MOF as a result of the hormonal effect which is a characteristic in male.

## CONCLUSION

Based on the current findings, it can be concluded that, metal organic frameworks have an impact on some physiological parameters, which was recorded by using FTIR technique. The data showed significant differences in all tested parameters in both treatments; however, Fe-MOF was more effective than Mn-MOF during all stages arising from larvae after application. It is worthy to mention that, these compounds can be used in control program against mosquitoes according to their acute and chronic effects.

## Conflicts of interest

There are no conflicts to declare.

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